

## RESEARCH ARTICLE

# Antibacterial Activity of Eel (*Anguilla* spp.) Mucus against *Salmonella typhi*

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Received date: Jul 23, 2016; Revised date: Aug 23, 2016; Accepted date: Nov 16, 2016

## Abstract

**BACKGROUND:** Typhoid fever has become one global health problem. Typhoid fever is caused by a Gram-negative bacterium, *Salmonella typhi*. Eel (*Anguilla* spp.) is a fish which lives in the sea or freshwater. Several previous studies have found that *Anguilla* spp. mucus has the ability as antibacterial against Gram-positive and negative. Although the antibacterial activity of *Anguilla* spp. against various pathogens had been reported, very little is known about its activity against *Salmonella typhi*. The aim of this study was to investigate antibacterial activity of *Anguilla* spp. mucus against *Salmonella typhi* bacteria.

**METHODS:** Present study was an experimental *in vitro* test. Antibacterial activity assays were carried out by the disc

diffusion method. Antibacterial activity was determined by the clear zone formed around the paper disc and minimum inhibitory concentration was determined by observing the lowest concentration which could inhibit the growth of *Salmonella typhi*.

**RESULTS:** Result of the present study showed that the *Anguilla* spp. mucus has inhibitory effects against *Salmonella typhi*. Minimum inhibitory concentration from the *Anguilla* spp. mucus was 12.5%.

**CONCLUSION:** *Anguilla* spp. mucus has antibacterial activity against the *Salmonella typhi* bacteria.

**KEYWORDS:** eel fish mucus, *Anguilla* spp., antibacterial activity, *Salmonella typhi*

*Indones Biomed J.* 2016; 8(3): 179-82

## Introduction

Typhoid fever is a non-communicable disease that has become a global health problem. WHO has reported that approximately 17 million cases of typhoid fever and 600,000 associated deaths occur each year. In Indonesia, one of the typhoid endemic areas, is found an average of 900,000 cases typhoid fever each year with over than 20,000 deaths.(1) Typhoid fever is caused by a Gram-negative bacterium, *Salmonella typhi* (*S. typhi*). The transmission of the bacteria usually from ingestion of contaminated food and water.(1,2)

Indonesia is one country that has potential aquatic resources where eels living. Eel (*Anguilla* spp.) is a fish living in the sea or in freshwater.(3) *Anguilla* spp. is one type of fish that produce mucus. The mucus layer on the surface of fish usually serves as the first line of defense against invasion between fish and its environment. *Anguilla* spp. mucus contains anti-microbial compounds such as antimicrobial peptide, lysozyme, lectins and protein. Several studies suggest that the mucus of *Anguilla* spp. has antibacterial activity against pathogenic bacteria, both Gram-negative bacteria such as *Edwardsiella tarda*, *Aeromonas* sp, *Aeromonas hydrophila*, *Escherichia coli* and Gram-positive bacteria such as *Micrococcus leteus*,

*Streptococcus difficile*.(4-6) Although the antibacterial activity of *Anguilla* spp. against various pathogens has been reported earlier, very little is known about its activity against *S. typhi*. Therefore, the present study aimed to investigate antibacterial activity of *Anguilla* spp. mucus against *S. typhi* bacteria.

## Methods

### Sample Collection

Sample was collected from the Lasolo River, in South Konawe area, South-east Sulawesi, Indonesia by fishermen and was identified by laboratory testing in Faculty of Fisheries, Halu Oleo University. Based on the morphology and zoological taxonomy, the sample was confirmed as *Anguilla* spp. by zoologist. Sample was kept in container that contains fresh water to increase the mucus production. The mucus was taken from the surface of eel's body by using plastic spatula and was collected into a sterile bottle for about 10 ml. *Anguilla* spp. mucus was made in various concentrations of 100, 50, 25, 12.5 and 6.25%. *Anguilla* spp. mucus that had not been diluted was expressed as mucus which has a concentration of 100%, while the other concentrations were prepared by diluting distilled water using the pipetting technique.

### Bacterial Strain

*S. typhi* was obtained from the collection of microbiology laboratory in Faculty of Medicine, Halu Oleo University, Indonesia. Before the tested bacteria were inoculated, Gram staining was done to make sure that the bacteria tested were not contaminated with other bacteria (Figure 1). The result concluded that the bacteria were *S. typhi*.

### Antibacterial Activity Assays

Antibacterial assays were carried out by the disc diffusion technique. Clear inhibition zones around the paper discs

indicated the presence of antibacterial activity of *Anguilla* spp. mucus. Minimum inhibitory concentration was determined by observing the lowest concentration that can inhibit the growth of *S. typhi* after incubated for 24 hours at 37°C.(7) Antibacterial activity assays were performed for three replicates. Ceftriaxone was used against pathogenic bacteria as positive control, while distilled water as negative control.

## Results

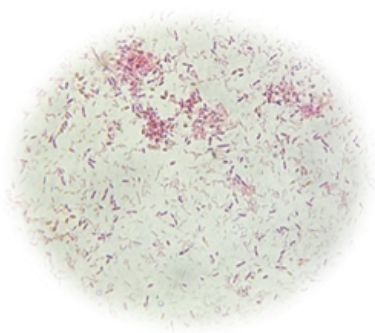
The mucus collected from *Anguilla* spp. showed inhibition in the growth of *S. typhi* (Table 1 and Figure 2) from antibacterial activity assays. The mucus collected from *Anguilla* spp. showed inhibition in the growth of *S. typhi* bacteria. Clear inhibition zones around the paper discs indicated the presence of antibacterial activity. Maximum inhibition zones was observed in concentration of 100% (42.2±5.4 mm), 50% (32.0±5.9 mm), 25% (28.3±1.7 mm), and 12.5% (21.0±4.8 mm). While in concentration of 6.25% the mucus of *Anguilla* spp. did not show any inhibition zones, so did the negative control (distilled water). Positive control (Ceftriaxone) showed a zone of inhibition for 48.1±2.2 mm.

**Table 1. Antibacterial activity of the *Anguilla* spp. mucus (values are the mean of three replicates±SD).**

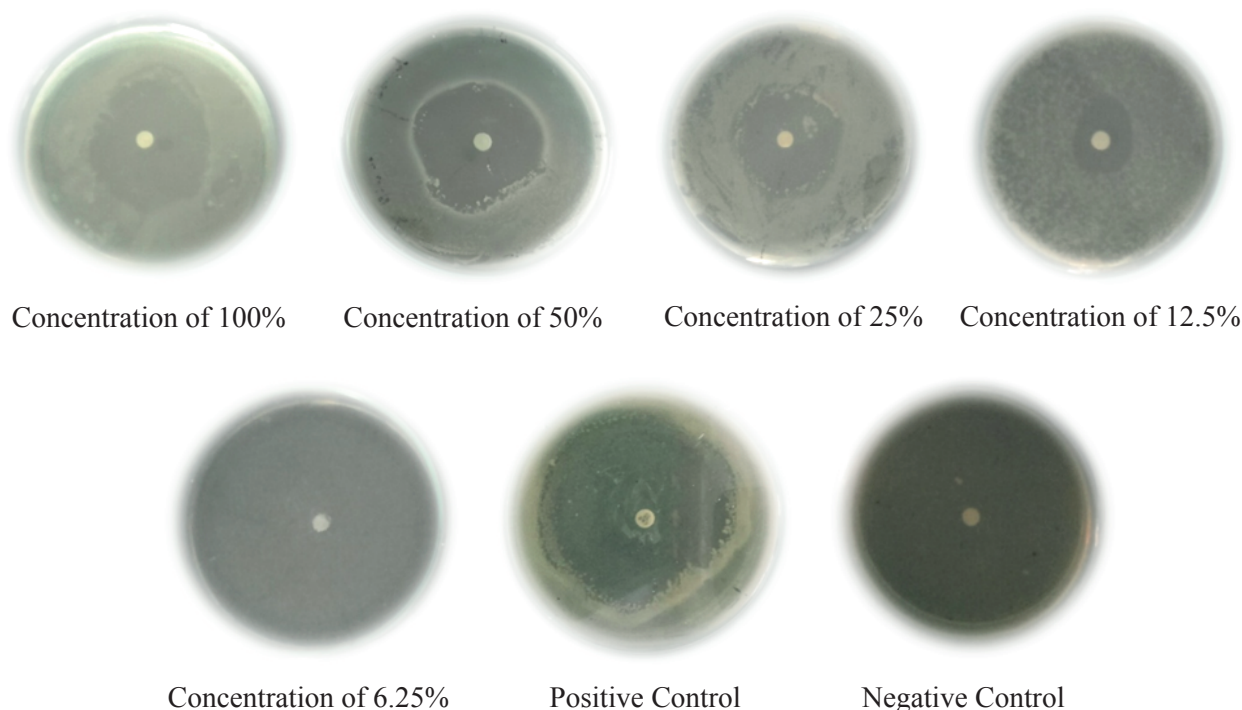
Treatment (concentration in %)	Inhibition zones (mm)
100	42.2±5.4
50	32.0±5.9
25	28.3±1.7
12.5	21.0±4.8
6.25	0
Ceftriaxone (positive control)	48.1±2.2
Distilled water (negative control)	0

## Discussion

The emergence of multi-drugs bacterial resistance in many endemic areas of *S. typhi* should be reduced.(1) The reports on the antibacterial activity of natural products against the pathogens are promising to be developed. Present study



**Figure 1. Microscopic image of Gram staining bacteria at 100x magnification.**



**Figure 2. The comparative antibacterial activity of *Anguilla* spp. mucus with negative and positive control.**

was carried out to investigate the antibacterial activity of *Anguilla* spp. mucus against *S. typhi*. In our study, we found that *Anguilla* spp. mucus has potential activity against *S. typhi*. *Anguilla* spp. mucus has strong ability to inhibit the growth of *S. typhi* bacteria in the concentration of 100 % and minimum inhibitory concentration in the concentration of 12.5%.

The epidermal layer of fish contains specialized glandular cells that produce mucus. The mucus layer of fish contains a variety of materials such as lectin, lysozyme and protein which has antibacterial activity that provide a first line of defense system against invading bacteria.(8,9) It has been reported that protein extracted from fish's epidermal mucus acts as antibacterial by a pore formation in cell membranes bacteria.(10)

Mucus of *Anguilla Japonica* has been known to contains two lactose-specific lectins, which are *Anguilla japonica* lectin (AJL)-1 and AJL-2, that act as antibacterial by agglutinate and suppression growth of both Gram-positive and Gram-negative bacteria.(5,9) Lectin has important role in innate immune defense by activation of the classical complement pathway.(11) It has been reported that mucus of *Anguilla japonica* contain AJN-10, a heat-tolerant and hydrophilic peptide, showed strong antibacterial activity against *Aeromonas hydrophila*.(4) Thus, skin mucus of eel needs to be characterized further, and can be explored as a potent antibacterial against *S. typhi*. Further study is needed

to explore the bioactive molecule and its mechanism responsible for antibacterial against *S. typhi* bacteria.

## Conclusion

*Anguilla* spp. mucus has an ability to inhibit the growth of *S. typhi*, because of its antibacterial activity against the *S. typhi* bacteria. Thus, this study suggests that *Anguilla* spp. mucus is a promising biopharmaceutical compound that can be effectively used against the *S. typhi* bacteria.

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